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The first record of *Neurospora tetrasperma* (anam. *Chrysonilia tetrasperma*) on *Platanus orientalis* in Iran

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Abstract — Sexual reproduction by *Neurospora tetrasperma* is reported and illustrated from Iran on *Platanus orientalis* in nature, the first report from Iran. It was found on a new host for this fungus.

Key words — teleomorph, morphology, taxonomy, fungi

Introduction

Shear & Dodge (1927) introduced the generic name *Neurospora* for four species characterized by dark ascospores with a grooved surface and longitudinal ribs. Numerous species were later added to the genus by others (Tai 1935, Gochenaur & Backus 1962, Nelson et al. 1964, Frederick et al. 1969, Mahoney et al. 1969, von Arx 1981, Perkins & Raju 1986, Krug & Khan 1991). Heterothallic/pseudohomothallic *Neurospora* species have been distinguished in the past by the morphological and biological species concepts (Turner et al. 2001, Dettman et al. 2003a, b). When *N. crassa*, *N. sitophila*, *N. intermedia* and *N. tetrasperma* were described in 1927 and 1935, intra- and interspecific crosses showed clear differences that influenced the authors of the descriptions (Shear & Dodge 1927, Tai 1935) well before the biological species concept was published in 1942 (Mayr 1942). Although morphology was the basis of the descriptions of the four species cited above, reproductive isolation measured by mating success has been regarded as a more reliable method for the identification of heterothallic

Neurospora (Perkins & Raju 1986, Perkins & Turner 1988, Shear & Dodge 1927, Tai 1935). Morphology nonetheless continues to be useful for identifying *N. tetrasperma* because this pseudohomothallic species produces perithecia with asci containing four dikaryotic and binucleate spores, as opposed to the eight-spored asci found in all other *Neurospora* species (Turner et al. 2001).

Morphological species recognition (MSR) is the dominant method of species recognition because it is an integral element of the description of every species and can be applied to most eukaryotic organisms (Dettman et al. 2003b). If sexual reproduction can be assessed, biological species recognition (BSR) using mating tests may be used to designate reproductively isolated biological species sensu Mayr (1942). However, the relationship between mating behavior in the laboratory and the potential to interbreed in nature often is unclear, and sexual activity has not been observed in nature or the laboratory for approximately 20% of the fungal kingdom (Hawksworth et al. 1995). Phylogenetic species recognition (PSR) accepts additional genetically isolated species that had not been recognized previously due to the lack of taxonomically informative morphological characters (phenotypic simplicity or plasticity) or incomplete reproductive isolation among species (Taylor et al. 2000). Typically, a single morphological or biological species with a cosmopolitan distribution is found to be composed of multiple cryptic, phylogenetic species that often are geographically distinct. In addition, PSR is applicable to all organisms, including those that cannot be induced to mate in the laboratory, as is required for BSR. For these reasons, PSR is becoming more popular for differentiating species, especially among mycologists, and is challenging BSR as the method of choice (Taylor et al. 2000).

Recently PSR of outbreeding *Neurospora* individuals has identified at least 15 genetically isolated, species-level clades, where previous BSR using mating to tester strains had delimited just five reproductively isolated species (Dettman et al. 2003a, 2006, Turner et al. 2001). These 15 phylogenetic species (PS) are referred to two sister clades. The first comprises four of five described species — *N. crassa*, *N. sitophila* (Shear & Dodge 1927), *N. intermedia* and *N. tetrasperma* (Tai 1935) — and three new *Neurospora* species tentatively labeled PS 1, 2 and 3 (Dettman et al. 2003a); Villalta et al. (2009) recently described the new species as *N. hispaniola* (PS1), *N. metzenbergii* (PS2) and *N. perkinsii* (PS3). The second clade comprises the fifth described species — *N. discreta* (Perkins & Raju 1986) — and seven new as-yet undescribed *Neurospora* species tentatively labeled PS 4–10 (Dettman et al. 2006).

Fungi have not been extensively investigated in Iran, and most reports of new taxa are limited to check lists without detailed descriptions. However, fungi of Iran have received more attention in the past few decades. The recent compilations by Ershad (1995) and Abbasi & Aliabadi (2009) of all available

reports on fungal species from different substrates lack any past observation of a *Neurospora* species from Iran.

Materials and methods

The plant material for this investigation was obtained from Gorgan, Golestan province, in the northeast of Iran in the summer of 2008. At the time of sampling, fire burned twigs of plane trees (*Platanus orientalis*) having fungal fruiting bodies on the surface were collected. There were multiple *N. tetrasperma* samples on several twigs from several burnt trees. Specimens of the fungus fruiting bodies were studied in the laboratory using an Olympus light microscope (model BH2). Handmade, thin sections of fruiting bodies were prepared using razor blades and morphological features of the fungus were studied. Also, pieces of plant tissue were placed on water agar (2%) medium after disinfestations and pure fungal cultures were obtained by transferring hyphal tips. Fungal isolates were grown on potato dextrose agar (PDA) culture medium at 25°C under continuous dark condition. Morphological characteristics of sexual and asexual stages of the fungus, as well as growth rates of fungal isolates, were studied. The fungal isolates were identified (based on morphological species concept) by comparison to the descriptions of Perkins & Turner (1988), Turner et al. (2001), and Garcia et al. (2004).

Results

The growth rate of fungus, as measured on PDA at 25°C under continuous dark conditions for 13 hrs, was 0.38 to 0.46 cm/hour. The margins of fungal colonies were smooth or partly irregular. Colony color was white initially, but subsequently, turned to pale orange. The colony expanded quickly and the expanding hyphae were broad, septate, thick-walled, hyaline and branched. Sporodochial tufts, observed only on the plant tissues, were orange in color (Fig. 1A and 1B) with dimensions of 1.2–7.5 × 0.65–2 × 0.32–1.2 mm and were composed of repeatedly branched conidiophores. Conidiophores were at first non-septate and without constrictions, but soon became swollen and septate (Fig. 1C). Conidia were produced at basipetal succession (Fig. 1C). Arthroconidia were subglobose to obovoid, smooth, 8–17 (12.6) × 6–11 (10.5) µm in diameter (Fig. 1E), and appeared orange color in mass. Arthroconidia became swollen and easily separated from each other by dehiscence of the wall and excretion of protoplasmic strands through the central pore of the (double) septa (Fig. 1D). The sexual stage of the fungus was formed abundantly on the culture medium (PDA) and the perithecia were visible on the culture medium and plant tissues (under the bark of tree) as very small black dots (Fig. 2A). Descriptive characteristics of sexual stage of fungus corresponded to those reported by Perkins & Turner (1988), Turner et al. (2001) and Garcia et al. (2004). Perithecia were superficial to somewhat immersed, scattered to aggregated, globose or subglobose with one prominent papilla (Fig. 2B) with 40–70 (54) µm in length. Perithecia were ostiolate, pale brown to dark brown, smooth or

downy with loose hyphae (Fig. 2C), and 300–420 (370) µm in diameter. Asci were unitunicate, hyaline, cylindrical, thin-walled having ring-like thickening at the tip, short stalked, often 4-spored (Fig. 2D), rarely 3-spored and 125–187 (160) × 15–19 (16) µm in diameter. Ascospores were uniseriate (Fig. 2D), one celled, ellipsoidal or elongated, initially hyaline, becoming yellowish brown to dark brown, ascospore wall surface with longitudinal and sometimes branched ribs (Fig. 2E). Ascospores were 22–41 (32) × 13–19 (16) µm in diameter and had circular and apical germ pores at each end. Based on morphological features of anamorphic and teleomorphic stages of the fungus on host plant and culture media, it was identified as *Neurospora tetrasperma* Shear & B.O. Dodge (anam. *Chrysonilia tetrasperma* (Shear & B.O. Dodge) Arx).

Specimen examined— IRAN, Golestan Prov., Gorgan city, near the Varsan village, on fire burned twigs of *Platanus orientalis* L. (*Platanaceae*), 36.835187 "N, 54.317329 "E, 10-VI-2008, Co. AGHAPOUR B (NEU 1154).

Discussion

Neurospora tetrasperma and its related anamorph, *C. tetrasperma*, are reported as new taxa for the mycoflora of Iran. According to the description of Jacobson et al. (2006), the fungus, *N. tetrasperma* (anam. *Chrysonilia tetrasperma*) has been reported in Europe (France and Portugal) from fire burned vegetation. We also looked at the Perkins collection, present at the Fungal Genetic Stock Center (FGSC), with over 4000 *Neurospora* isolates collected by David Perkins worldwide; there were reports of *N. tetrasperma* from Asia (India, Borneo, Indonesia and Malaysia), but none from the Middle East. Our results represent the first occurrence of a *Neurospora* species from Iran. Cannon et al. (1985) and Takeda et al. (2003) have reported *N. tetrasperma* on twigs and leaves of gorse (*Ulex* sp.) and maté (*Ilex paraguariensis* A. St.-Hil.). Our report is a first report on the new host plant, plane tree (*P. orientalis*) in the world.

There are no obvious morphological differences between the reproductive structures of our isolates compared with those described by Perkins & Turner (1988), Turner et al. (2001), and Garcia et al. (2004), except their dimensions, which could be attributed to different hosts and environmental conditions. The *Neurospora* species and their related *Chrysonilia* anamorphs can often be found on the surface following forest or grass fires, as our isolates were obtained from fire burned twigs. Perithecia have been reported under the bark of fire-injured trees (Kitazima 1925, Jacobson et al. 2001), in epidermal tissues of sugar cane stubble (Pandit & Maheshwari 1994, 1996), and on discarded corncobs carrying the yellow ecotype of *N. intermedia* (Pandit et al. 2000). Sexual fruiting bodies have rarely been found in nature at forest fire sites or elsewhere (Jacobson et al. 2003, Pandit & Maheshwari 1996, Perkins 2002). Sexual structures may not have been observed more frequently in the nature

Fig. 1 A–E. Asexual stage of *Neurospora tetrasperma* on *Platanus orientalis* burnt branch: A and B– sporodochia tufts; scale bar= 2 mm. C– conidiophores; scale bar= 100 µm. D– excretion of protoplasmic strands; scale bar= 10 µm. E– conidium; scale bar= 10 µm.

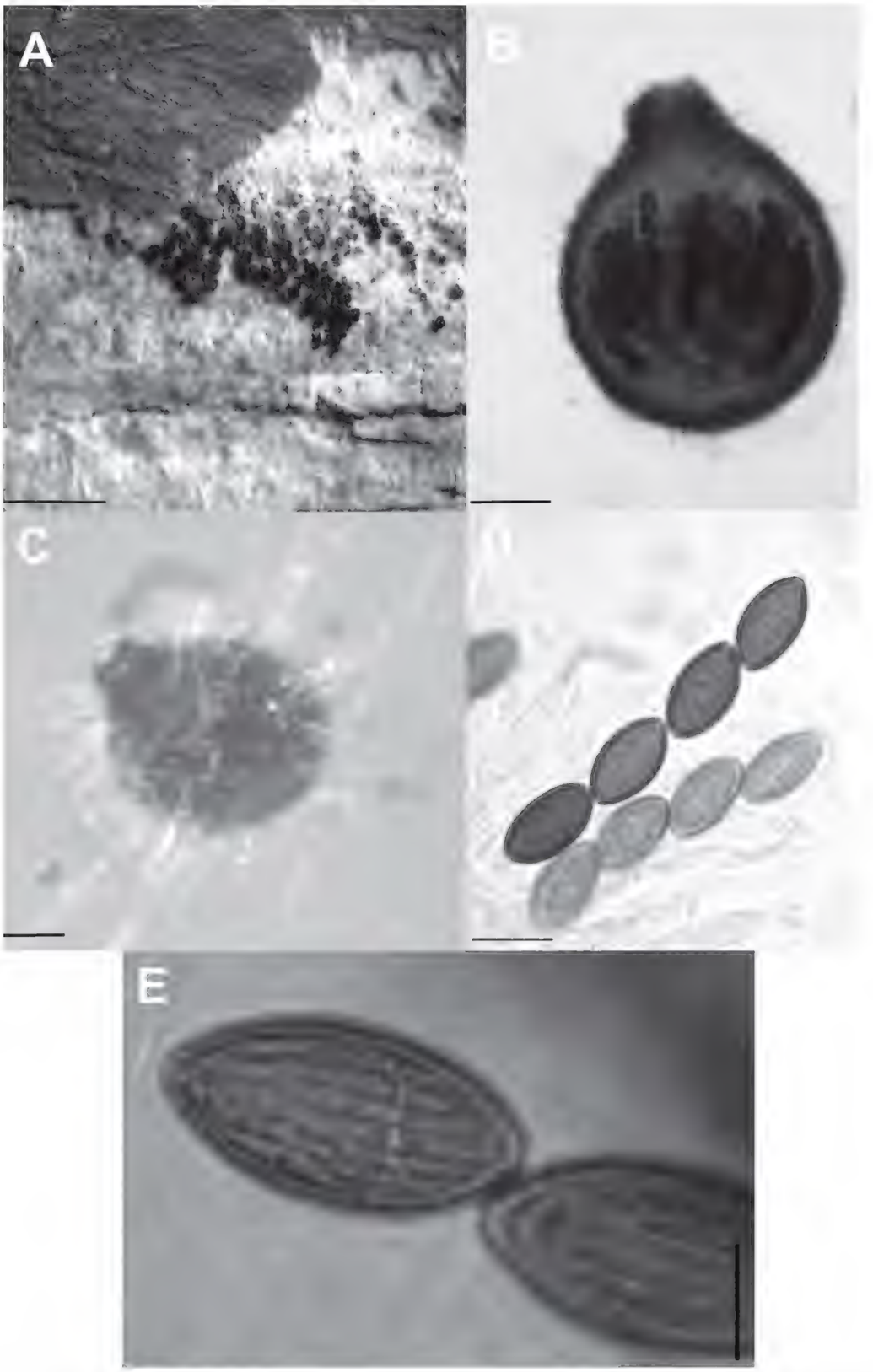

Fig. 2 A–E. sexual stage of *Neurospora tetrasperma* on *Platanus orientalis* burnt branch; A– perithecia scattered to grouped under the bark of *Platanus orientalis;* scale bar= 2 mm. B–perithecia with papilla; scale bar= 100 µm. C– loose hyphae around perithecia; scale bar= 100 µm. D– Asci 4-spored; scale bar= 20 µm. E– ascospore wall with longitudinal ribs; scale bar= 10 µm.

because of difficulty in recognizing black perithecia on burned substrate or by the perithecia being hidden within the substrate or delay of sexual reproduction until conidial blooms have dispersed (Turner et al. 2001). The role of resistant sexual ascospores in survival, dissemination, and mode of colonization is far from clear (Jacobson 2003). Jacobson et al. (2004, 2006) have observed that *Neurospora* is found growing beneath tree bark in western North America while in Europe it grows on the surface of tree bark. We observed perithecia under the bark of fire burned twigs. It seems that this is the first finding of *N. tetrasperma* perithecia in nature (D.J. Jacobson, pers. comm.).

In the past decade PSR has become a popular alternative to MSR and BSR (Taylor et al. 2000). Recently Menkis et al. (2009) applied both phylogenetic and biological species recognition to a collection of strains representing the geographic and genetic diversity of *N. tetrasperma* and were able to confirm a monophyletic origin of *N. tetrasperma*. Furthermore, they found nine phylogenetic species within the morphospecies and there was a high congruence between the phylogenetic and biological species recognition. Villalta et al. (2009) showed that PSR alone is powerful and accurate it also is important to the formal description of new species to account for reproductive isolation, biogeography, and morphology. We hope to continue our studies on phylogenetic and biological species diversity among reported *N. tetrasperma* strains in Iran in addition with some other collected strains of the fungus from other plants in the future.

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